Dear Editor,

Circulating mir-208a fails as a biomarker of doxorubicin-induced cardiotoxicity in breast cancer patients

Doxorubicin (DOX) is the first-line drug in the treatment of breast cancer. Despite its beneficial therapeutic effects, cardiomyopathy and heart failure are observed when DOX is chronically administered for several weeks (Octavia et al. 2012). Although several cardioprotective therapies have been proposed, cardiotoxicity remains a major concern of oncologists in cancer therapeutic practice (Fadilioglu et al. 2003; Fadilioglu et al. 2004; Alpsoy et al. 2013a, 2013b). Among all biomarkers of myocardial injury, cardiac troponins are the most used for its good sensitivity and integration in hospital routine. However, cardiac troponins are released in plasma only after membrane and tissue damage has occurred. Therefore, the development of new approaches to detect the cardiotoxic effects of DOX in the earlier stages is still required.

Recent studies have suggested that circulating mir-208a (a heart-specific microRNA) may serve as useful earlier biomarker of heart injury and drug-induced cardiotoxicity (Oliveira-Carvalho et al. 2013; Liu et al. 2014; Xiao et al. 2014; Nishimura et al. 2015). However, the potential of mir-208a as a biomarker of DOX-induced cardiotoxicity in clinical samples was not yet elucidated.

In brief, 59 female patients with breast cancer under the first round of chemotherapy with doxorubicin were enrolled in this study. Patients received a cumulative dose of doxorubicin (60 mg m⁻²; Bergamo), cyclophosphamide (600 mg m⁻²; Baxter) and paclitaxel (80 mg m⁻²; Blau) or docetaxel (75 mg m⁻²; Glenmark) during 3, 6, 9 and 12 weeks of treatment. Radiotherapy was indicated only after the end of the treatment; information about the surgery is not available. Cardiac troponin I (cTnI; assessed about the surgery is not available. Cardiac troponin I (cTnI; assessed in serial samples (0, 3, 6, 9 and 12 weeks). Patients that developed cardiotoxicity were grouped and compared with patients that did not. The study protocol was approved by the ethics committee at Faculdade de Medicina da Universidade de São Paulo and complies with the Declaration of Helsinki. Informed consent was obtained from each patient.

Peripheral blood was collected in 4-ml tubes with EDTA and centrifuged at 2000 g for 15 min at 4°C. Plasma samples were transferred to 1.5-ml microtubes and stored at −70°C. Small RNAs (>200 nt) were isolated from 200 μl of plasma by a miRNeasy Plasma/Serum kit (Qiagen). Cel-miR-39 was spiked in prior RNA isolation for data normalization. Reverse transcription was performed with a TaqMan MicroRNA Reverse Transcription Kit (Life Technologies) using a fixed volume of 5 μl of RNA sample as an input. Circulating levels of mir-208a were measured by RT-qPCR in triplicate using TaqMan MicroRNA Assays (Life Technologies) inputting 1.33 μl of cDNA. RT-qPCR reactions were plotted in plates of 384 wells and measured in QuantStudio 12 K Flex (Life Technologies). A heart tissue sample was also included as a positive control. All reactions were performed according to the manufacturer’s protocol.

As a result, seven patients (11, 86%) developed cardiotoxicity. Serum levels of cTnI were increased from 6.3 (±0.2) to 31 pg ml⁻¹ (±3.2) in the non-cardiotoxicity group whereas from 8.3 (±1.9) to 123.4 pg ml⁻¹ (±10.0) in cardiotoxicity patients. The serum levels of cTnl ≥ 40 pg ml⁻¹ (reference value) indicate myocardial injury. A decrease in the left ventricular function evaluation from 65.71 (±1.97) to 56.80 (±6.59) was also observed in cardiotoxicity patients whereas it was preserved in non-cardiotoxicity patients.

In contrast, miR-208a was not detected in any sample from both the groups even at a troponin peak at 12 weeks. The positive control (heart tissue sample) was amplified as expected. A new round of RNA isolation, reverse transcription and RT-qPCR was performed to confirm the results. miR-1 was also included as an endogenous positive control. However, mir-208a remained undetectable whereas miR-1 amplified in all samples showing the miRNA viability and absence of inhibitors.

Although previously published studies have suggested miR-208a as a heart injury biomarker (Liu et al. 2014; Xiao et al. 2014; Nishimura et al. 2015) these results clearly show that mir-208a was not circulating in the bloodstream of breast cancer patients with DOX-induced cardiotoxicity. In fact, these studies evaluated circulating levels of miR-208a under acute conditions. However, Nishimura et al. (2015) showed that after a single administration of a high DOX dose in rat the circulating level of miR-208a as well as cardiac troponins (cTnl and cTnT) did not change significantly whereas miR-1, miR-133a/b and miR-206 were increased. These results suggest that the toxic acute effect of DOX occurs apparently in skeletal muscle prior to myocardial damage.

Indeed, in a study conducted by Vacchi-Suzzi et al. (2012), the expression level of miR-208a in mice hearts decreases during the DOX treatment (cumulative doses) similarly with its encoding gene Myh6. In parallel, miR-208b and Myh7 increase their levels indicating a myosin switch that is associated with pathological cardiac remodeling. These results support our findings and partially explain the lack of miR-208a in the bloodstream of patients with DOX-induced cardiotoxicity.

In light of these results, we believe miR-208a is not released into the bloodstream by the DOX-injured heart and, therefore, is not useful as a biomarker of DOX-induced cardiotoxicity in breast cancer patients. These results reflect the species differences in miRNA rearrangement indicating that what happens in rodents do not necessarily translate to the human clinical setting. However, the search for new target miRNAs is still required and should not be discouraged.

Competing Interests

The authors declare that they have no competing interests.

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References


Oliveira-Carvalho V, Carvalho VO, Bocchi EA. 2013. The emerging role of miR-208a in the heart. DNA Cell Biol. 32: 8–12.

